AMENDMENTS TO THE SPECIFICATION

Please amend the specification as shown:

Please delete the paragraph on page 5, line 7 and replace it with the following paragraph:

Fig. 2 shows a procedure 1 for preparing a cassette construct. Figure discloses SEQ ID NOS 6-7, respectively, in order or appearance.

Please delete the paragraph on page 5, line 23, and replace it with the following paragraph:

Fig. 13 shows a method for preparing pGEMA and pGEMAS. Figure 13B discloses SEQ ID NO: 1.

Please delete the paragraph on page 7, lines 10-22 and replace it with the following paragraph:

The cassette construct can be prepared via conventional techniques. Preferably, a spacer sequence having blunt ends is first prepared, as shown in Fig. 2. Further, linker sequences each having a noncohesive protruding end and a blunt end, which are referred to as "lone linkers," can be ligated to the both blunt ends of the spacer sequence as an adaptor sequence and as an inverted adaptor sequence. Examples of lone linkers include LL-Sse8387I, such as LL-Sse8387IA (5'-GAGATATTACCTGCAGGTACTC-3') (SEQ ID NO: 6) and LL-Sse8387IB (5'-GAGTACCTGCAGGTAATAT-3') (SEQ ID NO: 7) and LL-SalI, such as LL-SalIA (5'-ATTGACGTCGACTATCCAGG-3') (SEQ ID NO: 8) and LL-SalIB (5'-CCTGGATAGTCGACGTC-3') (SEQ ID NO: 9) (Ko, M. S. H. et al., Nucleic Acids Res., 18, pp. 4293-4294, 1990). LL-Sse8387IA,B or LL-SalIA,B is phosphorylated and annealed. The annealed LL-Sse8387I or LL-SalI is ligated to a spacer sequence having blunt ends. The thus prepared cassette construct can be amplified via PCR using, for example, LL-Sse8387I or LL-SalIA as a primer.

Please delete the paragraphs on page 14, line 11 to page 16, line 21 and replace them with the following paragraphs:

(1) Preparation of pRNAi

An oligonucleotide having an arbitrary adaptor sequence and an inverted arbitrary sequence between which a spacer sequence can be inserted in the center is synthesized. An oligonucleotide (5'-GAGATATTACCTGCAGGTACTCACCCGGGTGAGTACCTGCAGGTAATATCTCA-3') (SEQ ID NO: 1) having a recognition sequence CCCGGG for the restriction enzyme *Sma*I in the center thereof and having A at its 3' end was synthesized herein, and this oligonucleotide was annealed (Fig. 13B), followed by cloning into a plasmid vector. Since protrusions (A) were generated at both 3' ends via annealing, the resultant was cloned into a commercially available TA cloning vector (the pGEM-T Easy Vector, Promega) (Fig. 13A) to prepare pGEMA (Fig. 13C). Further, a spacer sequence was inserted into the center of the cloned inverted repeat sequence (Fig. 13D to 13F). The center portion of the inverted repeat sequence of pGEMA was cleaved with the restriction enzyme *Sma*I (Fig. 13D), as a spacer sequence, a DNA fragment containing the intron sequence of the FAD2 genes from *Arabidopsis thaliana* was amplified via PCR (Fig. 13E, SEQ ID NO: 4), and the amplified fragment was inserted with the aid of T4 DNA ligase to prepare pGENAS (Fig. 13F). Subsequently, a region containing a cassette construct was cleaved with the restriction enzyme *Eco*RI, the recognition sequences of which are located at both sides of the cloning site of an oligonucleotide on the pGEM-T Easy Vector, and the cleavage product was inserted and ligated to the *Eco*RI cleavage site of the pBluescript II KS plasmid vector (Stratagene) with the use of T4 DNA ligase to prepare pRNAi comprising the recognition sequence for the restriction enzyme *Eco*RV at a position located outside of the adaptor sequence of the cassette construct (Fig. 14). Subsequently, the nucleotide sequence of pRNAi was examined via conventional techniques.

(2) Insertion of DNA fragment of target gene (target sequence) into pRNAi

A target sequence was inserted into a site outside one end of the cassette construct on pRNAi. Part of cDNA of a receptor protein kinase gene of zinnia (Z8755, DDBJ Accession No. AU293996, T. Demura et al., PNAS, 99, 15794-15799), which had been cloned into the pGEM-T Easy Vector (Invitrogen), was inserted into a site outside of the adaptor sequence. At the outset, a set of primers corresponding to the sequence located outside of the cloning site on the

vector; i.e., a forward primer (TGTAAAACGACGGCCAGT) (SEQ ID NO: 10) and a reverse primer (CAGGAAACAGCTATGACC) (SEQ ID NO: 11), were used to amplify via PCR a DNA fragment containing cDNA of Z8755, and a partial DNA fragment having blunt ends was prepared using the restriction enzyme *Afa*I (SEQ ID NO: 5). The resultant was inserted and ligated to the restriction enzyme *Eco*RV cleavage site of pRNAi with the aid of T4 DNA ligase (Fig. 15).

(3) Preparation of a chimera gene having an inverted repeat sequence of a target sequence

A plasmid comprising a target sequence inserted therein was used as a template to conduct PCR with the use of a primer (primer A) designed based on the nucleotide sequence of a vector located immediately outside of the target-sequence-insertion site and a primer (primer B) designed based on part of a spacer sequence and a nucleotide sequence containing an inverted repeat sequence located on the side of the cassette construct opposite from the DNA insertion site. In such a case, the amount of primer A used was excessively large compared with that of primer B. Thus, a double-stranded PCR product comprising a target sequence and an inverted repeat sequence thereof separated by a cassette construct was prepared. This is considered to be via a lariat configuration resulting from temporary annealing between the adaptor sequence and the inverted adaptor sequence in a single single-stranded PCR product generated upon PCR or via a dimmer configuration resulting from temporary annealing between these adaptor sequences of 2 single-stranded PCR products, followed by elongation and PCR. As shown in Fig. 16, oligonucleotide RNAiF (CACCCCTCGAGGTCGACGGTATCGATAAGCTTGAT) (SEQ ID NO: 12) comprising a nucleotide sequence (CCCCTCGAGGTCGACGGTATCGATAAGCTTGAT) (SEQ ID NO: 2) located immediately outside the recognition site for the restriction enzyme EcoRV of pBluescript II KS and having at its 5' end a sequence for cloning into pENTR/D Topo Vectpr (Invitrogen) was used at a final concentration of 0.6 μmol/l as primer A. Also, oligonucleotide RNAiR (GATTGAGATATTACCTGCAGGTACTCACCCGGGTG) (SEQ ID NO: 3) synthesized from a complementary strand of the 3' end (CACCC) of the spacer sequence and the nucleotide sequence (GGGTGAGTACCTGCAGGTAATATCT) (SEQ ID NO: 13) of an inverted adaptor sequence was used at a final concentration of 0.6 μmol/l as primer B. The reaction was carried out in 50 μl of a reaction solution comprising about 2 ng of a plasmid (pRNAi-Z8755) comprising target sequence (a partial sequence of Z8755), 1 μl of thermostable DNA polymerase

(KOD-Plus-, TOYOBO), 5 μl of 10x buffer (TOYOBO), 2 μl of 25 mM $MgSO_4$ (TOYOBO), 5 μl of 2 mM dNTP (TOYOBO), 3 μl of 10 μM RNAiF, and 3 μl of 1 μM RNAiR, and thermal denaturation was carried out at 94°C for 2 minutes, followed by the reaction at 94°C for 15 seconds and at 68°C for 3 minutes and 30 seconds. Such PCR cycle was repeated 50 times.

Attachments

- Sequence Listing, paper form
- Sequence Listing, computer readable form
- Statement to Support Filing of Sequence Listing